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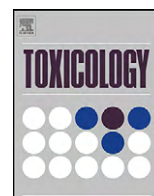




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### Review

## Post-translational modification by SUMO

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### ABSTRACT

Post-translational modifications (PTMs) are chemical alterations to a protein following translation, regulating stability and function. Reversible phosphorylation is an example of an important and well studied PTM involved in a number of cellular processes. SUMOylation is another PTM known to modify a large number of proteins and plays a role in various cellular processes including: cell cycle regulation, gene transcription, differentiation and cellular localisation. Therefore, understanding the role of SUMOylation in cell biology may allow the development of more efficient models, important in streamlining the drug discovery process. This review will focus on protein SUMOylation and its role in stem cell and somatic cell biology.

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### 1. Post-translational modifications

Post-translational modifications (PTMs) involve the addition of a chemical group following protein translation (Walsh et al., 2005). PTMs are essential for a variety of cellular processes and provide another level of protein regulation, which is usually reversible. There are a large number of PTMs that take place in the cell

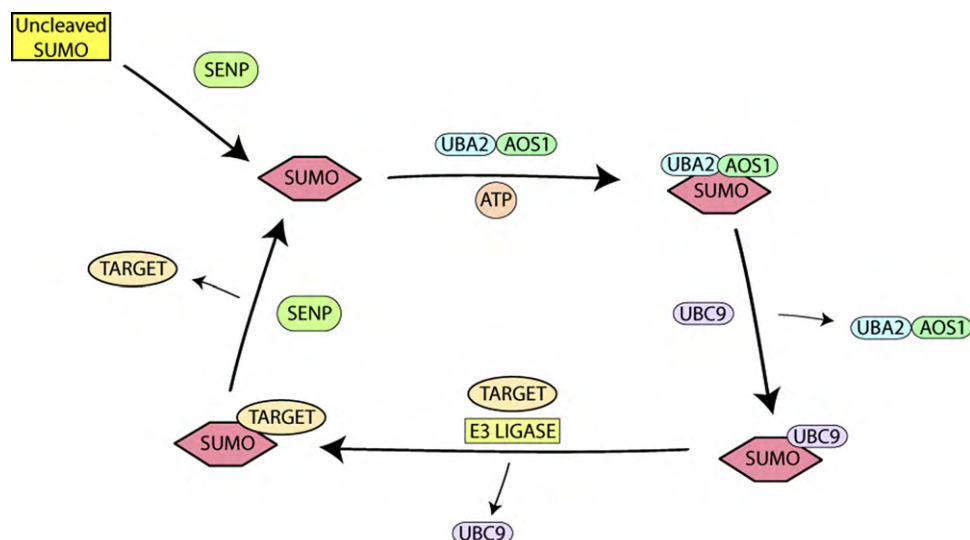
such as phosphorylation (Burnett and Kennedy, 1954), methylation (Grewal and Rice, 2004), acetylation (Glozak et al., 2005) and glycosylation (Spiro, 2002); regulating various biological activities such as transcriptional regulation (Waby et al., 2008) and protein degradation (Orford et al., 1997).

### 2. SUMO—small ubiquitin like modifiers

SUMOylation, another type of PTM, has a diverse range of effects within the living cell (Johnson, 2004). SUMO proteins are highly conserved in a large number of species and have been shown to be

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**Fig. 1.** The SUMO conjugation and deconjugation pathway. The SUMO conjugation pathway requires the activity of four enzymes: SENP the SUMO specific protease family; E1 composed of 2 subunits Uba2/Aos1; E2—Ubc9 and the E3 ligases. The reaction is initiated by cleaving the pro-form of SUMO at its C terminus, to its active state, by the SUMO specific proteases (SENPs). Following which, the E1 enzyme, a heterodimer of Uba2/Aos1, binds SUMO in an ATP dependant reaction, before the transfer to the E2 conjugating enzyme Ubc9. Ubc9 forms a reactive bond between itself and SUMO. Subsequently, the E3 ligase facilitates the transfer and covalent attachment of SUMO, via an isopeptide bond, to the target protein. This process is in equilibrium with the SUMO deconjugation (SENPs) machinery which cleaves the isopeptide bond.

important in many eukaryotic cell processes (Hayashi et al., 2002) including: cell cycle regulation, transcription, cellular localisation, degradation and chromatin organisation (Müller et al., 2001; Seeler and Dejean, 2003; Verger et al., 2003). Despite the name, SUMO only shares ~18% homology with ubiquitin (Müller et al., 2001) and is approximately 11 kDa in size, comparable to the 8 kDa ubiquitin molecule (Müller et al., 2001). SUMO has been found to bind to the lysine residue on the following consensus sequence;  $\psi$ KxE (where  $\psi$  corresponds to a large hydrophobic amino acid, K is a lysine residue, x is any amino acid and E is a glutamic acid residue) on the target protein. Three homologues exist in mammals, SUMO-1, -2 and -3. SUMO-2 and -3 share 95% homology with each other, but only share 50% identity with SUMO-1 (Johnson, 2004). SUMO-2 and -3 have the ability to form polySUMO chains, covalently binding to themselves via the lysine residue at the N terminus consensus motif  $\psi$ KxE. SUMO-1 lacks this consensus site and as a consequence is unable to form polychains (Kroetz, 2005) and acts as a polySUMO chain terminator (Ulrich, 2009).

### 3. SUMO conjugation

#### 3.1. Pathway overview

The SUMO conjugation pathway has a lot in common with the ubiquitination pathway. Both processes involve the use of three enzymes: E1: activating enzyme, E2: conjugating enzyme and E3: ligase (Fig. 1) (Takahashi et al., 2001). SUMO is bound to its target protein via an isopeptide bond formed between an  $\epsilon$ -amino group on the lysine residue on the target protein and the C terminal carboxyl group on the SUMO protein (Desterro et al., 1997). The pro-form of SUMO needs to be cleaved prior to protein conjugation. This is carried out by isopeptidases, also known as the SENP SUMO deconjugating enzymes (Mukhopadhyay and Dasso, 2007). The SUMO activating enzyme (E1), SAE1/2, commences the reaction process by interacting with SUMO (activated by SENP enzymes—Fig. 1), to form a high energy thiolester bond. The SUMO conjugating enzyme (E2) then binds SUMO via its cysteine residue in its active site. This intermediate provides a highly reactive species, important in the final conjugation, usually facilitated by an E3 ligase (Kroetz, 2005). SUMO E3 ligases act to either activate

Ubc9 or bring Ubc9 and the target protein within close proximity of each other, thus enhancing SUMOylation (Ulrich, 2009). They can be regarded as E3 enzymes as they are able to bind to the E2 and the substrate and facilitate the formation of the bond formed between SUMO and the target protein. It has also been shown that a large number of proteins (~40%) can be SUMOylated without the presence of the consensus sequence ( $\psi$ KxE), demonstrating differences in substrate specificity (Ulrich, 2009).

#### 3.2. The enzymes involved

##### 3.2.1. E1

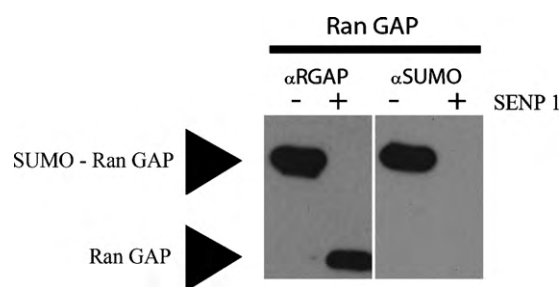
Unlike the ubiquitin (Ub) E1, the SUMO E1 exists as a heterodimer; with each monomer corresponding to a particular region of the Ub E1. The SAE subunit Aos1 (SAE1) shares similarity with the N terminus of the Ub E1, whilst Uba2 (SAE2), the second component of the SAE complex, is similar to the C terminus of the Ub E1 (Johnson et al., 1997). The monomers are never found individually and hence it is assumed that they are unable to function independently (Azuma et al., 2001). The SAE complex is responsible for preparing SUMO for transfer to the SUMO conjugating enzyme, Ubc9 (Walden et al., 2003).

##### 3.2.2. E2

Ubc9 is the only known SUMO conjugating enzyme, unlike the ubiquitination pathway where each E2 has a specific set of target proteins (Hayashi et al., 2002). Ubc9 contains an active site with a cysteine residue which is responsible for binding the SUMO molecule directly to the  $\psi$ KxE sequence found on the target protein (Sternsdorf et al., 1999).

##### 3.2.3. E3

In contrast to SUMO E2s, a larger number of SUMO E3 ligases have been discovered and have been categorized into three types: the protein inhibitor of activated STAT—signal transducer and activator of transcription (PIAS) family (Hochstrasser, 2001), the nuclear pore proteins Ran binding protein 2 and nucleoporin 358 (RanBP2/Nup358) (Pichler et al., 2002) and the polycomb group protein Pc2 (Kagey et al., 2003). E3 ligases are usually substrate specific with little redundancy found within the system.



**Fig. 2.** *In vitro* SUMOylation of RanGAP. An *in vitro* SUMO conjugation assay was carried out to SUMOylate the RanGAP protein. RanGAP was incubated with the SUMO conjugation machinery; SUMO1, SAE1/2 and Ubc9 under the following conditions: 10  $\mu$ l containing 50 mM Tris pH 7.5, 5 mM  $MgCl_2$ , 2 mM ATP, 5 mM DTT, 100 ng SAE2/1 (E1), 100–600 ng Ubc9 (E2), Sumo 2.5  $\mu$ g and substrate 1–3  $\mu$ g. The solution was incubated at 37 °C for 2.5 h and the reaction was stopped by the addition of LDS sample buffer. An additional control was added where the SUMO conjugated RanGAP is deconjugated using SENP 1. The conditions for the reaction are as follows: iodoacetamide was added to the conjugated RanGAP solution at 10 mM and incubated at room temperature for 30 min.  $\beta$ -Mercaptoethanol or DTT was added at 20 mM and left for 15 min at room temperature. SENP1 was finally added at 10 nM and incubated for 1 h at 37 °C. The reaction was stopped by the addition of LDS buffer. The samples were run on an SDS-PAGE gel and were then used to detect SUMO conjugation using Western blotting. The membranes were first probed for RanGAP (1:500) and as seen in this figure, an upper band is observed, a 10 kDa shift upwards; where free RanGAP is approximately 32 kDa and SUMOylated RanGAP is approximately 42 kDa. In the control lane with the addition of SENP1, the upper band disappears; suggesting RanGAP is SUMO modified *in vitro*. The same membrane was stripped and probed for SUMO 1 (1:1000), as shown in this figure; the upper band is also SUMO positive.

The largest group of E3 ligases are the PIAS proteins with four genes in mammals: PIAS1, PIAS3, PIASx and PIASy (Liu et al., 1998). The PIAS E3s have a conserved region consisting of a SAP domain responsible for binding AT rich DNA sequences and an SP-RING domain which binds to Ubc9 and promotes SUMOylation (Schmidt and Müller, 2002). They also contain SUMO interaction motifs (SIMs) that are able to directly bind SUMO (Rytinki et al., 2009). It has been found that the different PIAS proteins SUMOylate distinct sets of substrates, with occasional overlap (Schmidt and Müller, 2002). The second group of E3 SUMO ligases consists of the nuclear pore protein RanBP2 (Nup358) with only one known substrate, RanGAP1, a GTPase activating protein important in nuclear transport of proteins (Nishimoto, 1999; Saitoh et al., 1997). We have also shown that RanGAP can be SUMO modified *in vitro*, as displayed in Fig. 2. The final family SUMO E3 ligase identified so far is the PC2 protein part of the polycomb group (Kagey et al., 2003). Pc2 has been shown to SUMOylate the transcriptional co-repressor CtBP, localising it to the nucleus (Lin et al., 2003), and to co-localise with PcG bodies (Kagey et al., 2003).

### 3.3. Regulation of SUMO modification

SUMO modification is a dynamic process involving both conjugation and deconjugation enzymes. The deconjugation enzymes function by cleaving the isopeptide bond between SUMO and the modified protein (Melchior et al., 2003). There are seven isoforms of these isopeptidases, including SENP1, SENP2, SENP3, SENP6 and SENP7 (Mukhopadhyay and Dasso, 2007). The SENPs contain a Ulp domain at their C terminus responsible for cleaving the isopeptide bond and distinct N terminal domains that regulate their cellular localisation, suggesting each SENP has a distinct set of substrates (Mukhopadhyay and Dasso, 2007). In addition to their deconjugation role, the SENPs also play an essential role in maintaining the levels of free SUMO within the cell (Ulrich, 2009). Other forms of SUMO regulation include the E3 ligases and the presence of the consensus motif on target proteins. It has previously been stated that 40% of proteins modified by SUMO do not have the typical consen-

sus sequence; as such this could also be regarded as another form of regulation.

## 4. The role of SUMO conjugation in the cell

Over the last decade a number of groups have investigated how the SUMO pathway is regulated in response to different stimuli. In response to heat shock, erythroleukemia cells induce transcription of heat shock factor 1 (HSF1). After its translation, HSF1 is phosphorylated prior to its SUMOylation, which enhances its DNA binding ability (Hong et al., 2001). It is also widely recognised that SUMO alters protein activity by modulating other PTMs, such as phosphorylation and ubiquitination. For example, SUMOylation of I $\kappa$ B $\alpha$ , an important factor in the inflammatory response, prevents its ubiquitination, and therefore inhibits its degradation and subsequent NF- $\kappa$ B activation and nuclear translocation (Desterro et al., 1998). SUMO can also regulate protein activity by modulating its interactions with other macromolecules or proteins. Various models have been proposed such as the addition of SUMO by altering protein configuration, creating a new interaction motif affecting its function (Johnson, 2004). An interesting example of interaction motifs is arsenic induced RNF4 mediated degradation of promyelocytic leukemia (PML) bodies. In the presence of arsenic, PML is polySUMOylated, and following the recruitment of RNF4, an E3 Ub ligase, PML is ubiquitinated and degraded (Tatham et al., 2008).

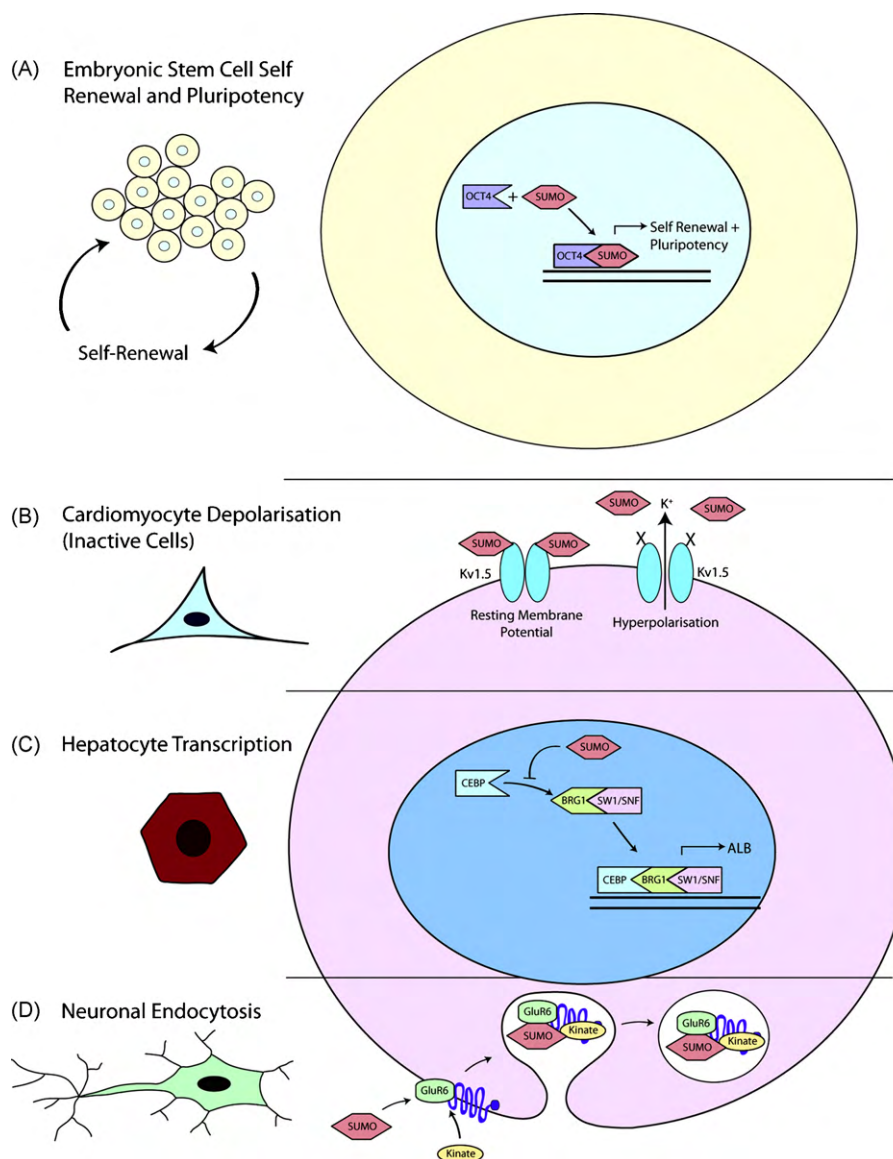
## 5. SUMO modification plays an important role in development and cell biology

Various studies have shown that disruption of the SUMO pathway causes abnormal cellular differentiation. Moreover, disruption of the SUMO pathway during embryogenesis may lead to embryo lethality (Nacerddine et al., 2005; Nowak and Hammerschmidt, 2006), demonstrating the requirement for SUMOylation during development. Due to the lethal nature of Ubc9 knock outs during development, other experimental strategies are necessary to determine the precise role of SUMOylation. *In vitro*, there has been a focus on the role of SUMOylation in a number of cell types, human embryonic stem cells (hESCs) and representatives of all three germ layers. These models, although not *in vivo*, provide a good developmental surrogate.

### 5.1. Human embryonic stem cells

Human embryonic stem cells (hESCs) are isolated from the inner cell mass of blastocyst stage embryos and are self-renewing cells capable of forming cell types from the three germ layers: mesoderm, endoderm and ectoderm (Fletcher et al., 2008). The ability to culture hESCs under standardized conditions and differentiate these cells into a variety of cell types using highly efficient and reproducible protocols may provide an inexhaustible resource for clinical and industrial application (Hannoun et al., 2010a,b; Hay et al., 2008). SUMO modification has been shown to have an important role in both hESC self-renewal and pluripotency (Wei et al., 2007). Oct4 is a POU transcription factor associated with the undifferentiated and pluripotent status of embryonic stem cells (Hay et al., 2004; Hardeland et al., 2002; Nichols et al., 1998). It is known to be SUMO modified, which results in its increased stability, DNA binding and transcriptional activity (Wei et al., 2007) (Fig. 3). Sex determining region Y box 2 (SOX2) is another important transcription factor required for embryonic stem cell self-renewal in an undifferentiated state (Cai et al., 2006). It was recently shown by Hoof and colleagues that SOX2 is SUMO modified as a result of phosphorylation. It has been suggested that SUMO modification of SOX2 affects its transcriptional activity (Hoof et al., 2009; Hietakangas et





**Fig. 3.** The effect of SUMO modification in various cell types. SUMO modification affects a number of cellular processes. (A) In human embryonic stem cells (hESCs), SUMO binds to Oct4 in the nucleus (blue), enhancing its stability and transcriptional activity which is an important regulatory mechanism in hESC self-renewal and pluripotency. SUMO modification also plays an important role in somatic cell biology. (B) In cardiomyocytes, SUMOylation regulates the properties of the Kv1.5 potassium voltage channel located at the plasma membrane. These channels play an essential role in cardiomyocyte membrane potential. The inhibition of SUMO modification to the Kv1.5 channel results in the opening of membrane channels, exporting potassium ions, which results in cellular hyperpolarisation. (C) SUMO conjugation in hepatocytes regulates the transcriptional activity of C/EBP impacting on albumin (ALB) expression within the nucleus (blue). SUMO modification of C/EBP inhibits its ability to form a complex with BRG1/SW1/SNF essential for high level albumin expression. SW1/SNF is a chromatin remodelling complex and BRG1 is a core subunit of the complex. (D) SUMOylation has also been shown to regulate the activity of the GluR6 receptor in neural cells by endocytosis. Kinase induced receptor internalisation on the cell plasma membrane is dependent on SUMO modification of GluR6, thus affecting neurone excitability.

al., 2006) but further investigation is required. The role of SUMOylation has also been determined in cell types representative of the three germ layers: endoderm, mesoderm and ectoderm.

## 5.2. Endoderm

The endoderm layer is formed during embryogenesis and is the precursor of liver, pancreas and lung amongst others (Tam et al., 2003). SUMOylation plays an important role in hepatocyte biology regulating C/EBP $\alpha$ , a crucial factor in hepatic differentiation (Pedersen et al., 2001; Sato et al., 2006). SUMOylation of C/EBP $\alpha$  prevents its association with BRG1, a core subunit in the SW1/SNF chromatin remodelling unit, leading to the inhibition of albumin expression (Sato et al., 2006) (Fig. 3). In line with this, it has been shown that there is a decrease in levels of SUMOyla-

tion as rat hepatocytes mature (Sato et al., 2006), suggesting an inhibitory effect of SUMOylation in hepatocyte terminal differentiation. The mitochondria are an essential component of hepatocytes, the main cell type in the liver, and are required for efficient liver function. Mitochondrial levels in the cell are dynamic and continuously undergo fusion and fission (Twig et al., 2008; Frazier et al., 2006). It has been shown that an increase in SUMO-1 expression results in an increase in mitochondrial fragmentation by stabilising the GTPase dynamin-related protein 1 (DRP1) (Harder et al., 2004). Further investigation of this pathway has revealed that SENP 5, a SUMO deconjugating enzyme, is required for normal mitochondrial morphology and levels of reactive oxidative species within the cell, partly by SUMO deconjugation of DRP1 (Zunino et al., 2007). In the pancreas, SUMO modification of islet cell autoantigen 512 (ICA512) has been shown to disrupt its binding to STAT5

and inhibit insulin and granule related gene transcription (Mziaut et al., 2006).

### 5.3. Mesoderm

During development the mesoderm differentiates into muscle, cartilage, bone, blood and connective tissue (Biggers and Borland, 1976). The heart significantly relies on the coordination of various ion channels for regular function. One such voltage gated channel is the potassium channel Kv1.5 found in atrial myocytes which modulate membrane potential of smooth muscle cells (Lagrutta et al., 2006). Benson and colleagues have shown that Kv1.5 has two conserved consensus SUMOylation motifs, which play an important role in hyperpolarisation (efflux of potassium ions) (Benson et al., 2007) (Fig. 3). At the initial stages of development, the polycomb 2 protein (Pc2), part of the polycomb repressor complex 1 (PRC1), is SUMOylated. This allows efficient complex formation and its recruitment to methylated histone 3 for controlled gene silencing. On mesoderm formation, SENP2 is recruited to PRC1, deSUMOylates the Pc2 protein and allows the expression of GATA4 and 6 transcription factors essential for normal cardiac formation (Kang et al., 2010). Interestingly, in adult cardiomyocytes, SUMO modification of GATA4 results in increased transcriptional activity, and promotes cardiogenic gene activity (Wang et al., 2004).

### 5.4. Ectoderm

Ectodermal differentiation results in the formation of the skin and nervous system (Pelton et al., 1998). SUMOylation also has a vital role in the nervous system. GluR6 is a highly expressed kainate receptor found in the brain, and is concentrated in the hippocampus (Nasu-Nishimura et al., 2010). The receptor is known to regulate neuronal excitability and as such is involved in learning, memory and synaptic plasticity (Barberis et al., 2008). It has been shown that the internalisation of the receptor upon kainate stimulation is regulated by SUMOylation (Fig. 3). GluR6 is internalised via kainate or *N*-methyl-D-aspartate (NMDA) induced endocytosis. Only kainate induced internalisation requires GluR6 SUMOylation. The mutation of the SUMO consensus motif in GluR6 results in a large reduction in kainate induced GluR6 internalisation and disrupts regular synaptic function (Martin et al., 2007). Another factor important for both brain development and neuronal differentiation is MEF2A. MEF2A and its associated family members have been shown to be involved in the proliferation, differentiation and apoptosis of cells found in the developing brain (McKinsey et al., 2002). SUMOylation of MEF2A decreases its transcriptional activity, suppressing Nur77 function; and promotes dendritic claw differentiation (Shalizi et al., 2006).

## 6. Conclusion

SUMOylation is an important PTM known to play roles embryonic stem cell and somatic cell biology. Given its importance in cell biology, it is critical that we understand SUMOylation in order to generate stable and high fidelity models that predict human drug toxicity. These models will not only be useful tools for toxicology, but will also provide a system whereby we can investigate the role(s) of SUMO modification in response to numerous stimuli. This will undoubtedly provide information on novel mechanisms of action with the possibility of developing new medicines and clinical investigation strategies.

### Conflict of interest statement

The authors report no conflict of interest.

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